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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE MULTI-COMPONENT ANALYSIS OF SOME IMPORTANT CATEGORIES OF ANTI-HYPERTENSIVE AGENTS

SYNOPSIS

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CHEMISTRY

Submitted by

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INTRODUCTION:

1.1 Introduction to Hypertension: Hypertension is a disease of elevated systolic and/or diastolic blood pressure (BP) characterized by increase in BP by 150 mm Hg and diastolic BP by 100 mm Hg. Hypertension is one of the leading cause of mortality in the world. In hypertension BP of arteries are constantly elevated. Hypertension leads to heart attack, stroke, peripheral vascular disease, loss of vision and kidney disease.

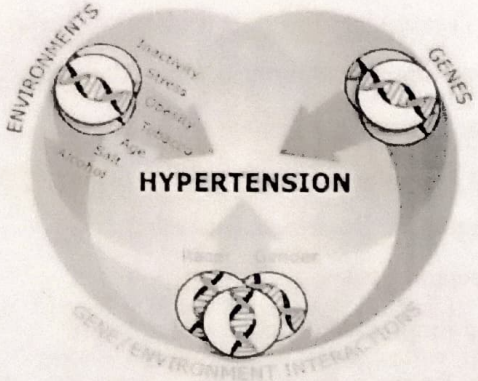

Figure 1: Causes of Hypertension

Increased BP develops slowly over many years usually without any specific identifiable cause. However, some possible medical causes, such as particular medications, kidney disease, adrenal problems or thyroid problems, must first be excluded. High BP that develops over time without a specific cause is considered benign or essential hypertension. BP also tends to increase as a person ages. A primary risk factor for pre hypertension is being overweight. Other risk factors include a sedentary lifestyle, eating high sodium foods, smoking and excessive alcohol intake. BP levels appear to be familial, but there is no clear genetic pattern.

1.2 Introduction of Antihypertensive Drugs: There is growing confirmation that high B.P. is a significant hazard feature, popular the progress of arterial fibrillation. The researchers established that the vulnerability of emergent arterial fibrillation reduced through 24 percentages with a 12mm mercury drop in systolic force following adjusting for period, sex and body mass and pulse pressure.

Medical researchers at the Erasmus University Medical School have discovered a natural mineral salt that significantly lowers blood pressure in people suffering from mild to moderate hypertension. The salt ***"Saga Salt"*** (Akzo Nobel, Netherlands) occurs naturally in Iceland and contains 41 percent sodium chloride, 41 percent potassium

chloride, 17 percent magnesium salts and 1 percent trace minerals. The researchers tested the salt in randomized double blind placebo controlled trial involving 100men and women aged 55 to 75 years. The participants had systolic blood pressures between 140 and 200mm Hg or diastolic pressures between 85 to 100mm Hg.

Commonly Use drugs include -

A. ***Diuretic***

Thiazide and related agents: Hydrochlorothiazide, Chlorthalidone

Loop diuretics: Frusemide, Ethacrinic acid, Bumetanide, Torsemide

Potassium sparing diuretic : Spironolactone, Amiloride, Triamterene

B. ***Drugs Acting on Sympathetic System***

Centrally acting: Clonidine, Gaunabenz, Guanfacine, Methyldopa

Catacolamine depleters: Reserpine, Guanadrel

Andrenergic receptor blocker

(i) β blockers: Propranolol, Atenolol, Metoprolol

(ii) α blockers: Prazosin, Phentolamine, Doxazosin, Terazosin, Phenoxybenzamine

(iii) Mixed blockers: Labnetalol, Carvediol

(iv) Imidazole receptor antagonist: Ritmenidine

C. ***Adrenergic neuron blocker:*** Gaunethidine

D. ***Ganglion blocker:*** Hexamethonium, Mecamylamine

E. ***Drug act by reflex mechanism:*** Veratrum alkaloid

F. ***Calcium channel blocker:*** Nifedipine, Amlodipine, Diltiazem, Felodipine, Nimodipine, Isrodipine, Nicardipine

G. ***Drug acting on rennin angiotensin system***

Drug blocks rennin release: Propanolol, Clonidine

ACE inhibitors: Captopril, Lisinopril, Ramipril

Angiotensin inhibitor: Saralasin

Angiotensin (Specific ATI) receptor blocker: Losartan, Valsartan, Candesartan Celexetil, Olmesartan Medoxomil, Irbesartan, Telmisartan

Aldoesterone inhibitor: Spironolactone

H. ***Vasodilator:***

Arterial: Hydralazine, Minoxidil, Diazoxide, Fenoldopam

Arterial and venous: Sodium nitropruside

I. Potassium channel activators and Nitric oxide donars such as Nitroglycerine, Nicorandil

1.3 Significance of Analytical Methods: Quality is important in every product or service, but it vital in medicine as it involves life. Unlike other consumer goods, there can be and there is no second quality.

Therefore analytical methods which are a measure of quality of the drugs play a very comprehensive role in drug development and follow up activities, to assure that a drug product meets the established standard, are a stable and will continue to meet purported quality throughout its shelf life.

Selected method must be validated from the point of discriminatory and susceptible to observe the identified and unidentified impurities also i.e. these methods should be validated.

1.4 High Performance Liquid Chromatography (HPLC): HPLC considering an advanced form of liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to understand better the role of individual molecules. More than 85% of general pharmaceutical are analysed by HPLC. The technique of chromatography was originally developed by Russian Botanist M.S.d Tswett in 1903 but after that, plenty of revolutions and amendments were done and it is till going on. In liquid chromatography, a mixture of molecules dissolved in a solution (mobile phase) is separated into its constituent parts by passing through a column of tightly packed solid particles (Stationary Phase). The separation occurs because each component in the mixture interacts differently with the stationary phase. Molecules that interact strongly with the stationary phase will move slowly through the column, while the molecules that interact less strongly will move rapidly through the column. This differential rate of migration facilitates the separation of the molecules.

HPLC utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector to signal the characteristic retention time for the analyte. Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent(s) used, and the flow rate of the mobile phase.

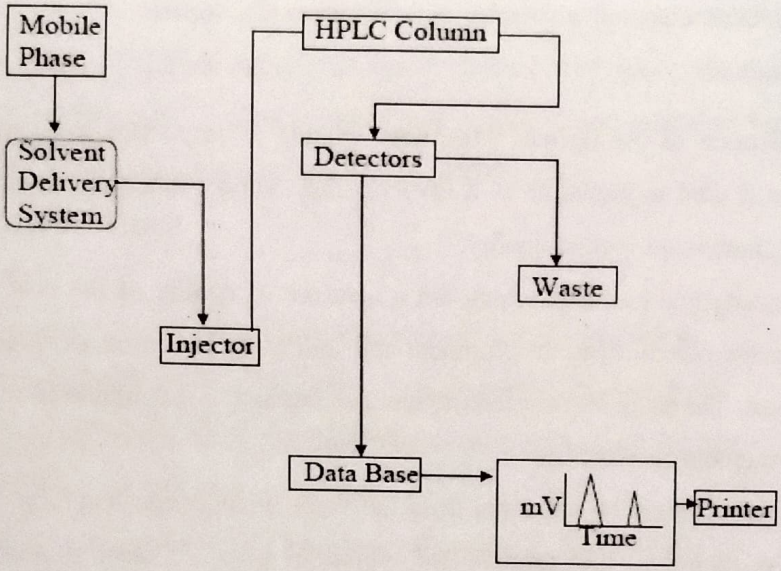

Figure 2: Block Diagram of HPLC System.

1.4.1 Method Development of HPLC: The broad assortment of apparatus, columns, eluents and equipped parameters involved creates method development by HPLC appear difficult. The procedure is prejudiced by the environment of the analyte and monitors the subsequent steps:

Step-1 Preliminary studies i.e. characteristics of drug with other components.

Step-2 Selection of early situations

Step-3 Exactness optimization

Step-4 Organization optimization

Step-1 Preliminary studies: When developing an HPLC method, the first step is always to consult the literature to know the physico-chemical properties of anlayte. The properties like solubility, polarity, molecular weight and partition coefficient are key parameters in selecting the right column and mobile phase for a successful chromatographic separation.

Sample Preparation: The sample matrix determines whether the sample requires dissolution, filtration, extraction, preconcentration or clean up. Chemical derivatization may be required to assist detection sensitivity or selectivity.

Types of Chromatography: Reverse phase is the choice for the majority of samples, but if acidic or basic analytes are present then reverse phase ion suppression (for weak acids or bases) or reverse phase ion pairing (for strong acids or bases) should be used. The

stationary phase should be C_{18} bonded. For low/medium polarity analytes, normal phase HPLC is a potential candidate, particularly if the separation of isomers is required.

Solvent System Selection-Isocratic/Gradient: This is only a requirement for complex samples with a large number of components (20-30) because the maximum number of peaks that can be resolved with a given resolution is much higher than in isocratic HPLC.

Gradient HPLC will also give greater sensitivity, particularly for analytes with longer retention times, because of the more constant peak with (for a given peak area, peak height is inversely proportional to peak width).

Detectors: UV detectors, single channel, multi channel or photodiode array are the most common detectors used in HPLC.

Fluorescence or electrochemical detectors are used for trace analysis. For preparative HPLC, refractive index is preferred because it can handle high concentrations without overloading the detector.

UV Wavelength: The analytical sensitivity is maximum at λ_{max}, but the wavelengths showing high absorbance can be used as analytical wavelengths. The UV wavelengths below 200nm are avoided because mobile phase interferences and detector noise increase in this region. Higher wavelengths give greater selectivity.

Step-2 Selection of Chromatography Conditions: This step determines the optimum conditions to adequately retain all analytes, that is, ensures no analyte has a capacity factor of less than 0.5 (poor retention could result in peak overlapping) and no analyte has a capacity factor greater than 10-15 (excessive retention leads to long analysis times and broad peaks with poor detectability). Selection of the following then required.

Mobile Phase Solvent Strength: The solvent strength is a measure of its ability to sweep analytes from the column. It is generally controlled by the concentration of the solvent with the highest strength; for example, in reverse phase HPLC with aqueous mobile phases, the strong solvent would be the organic modifier, in normal phase HPLC, it would be the most polar one. The aim is to find the correct concentration of the strong solvent.

Step-3 Selectivity Optimization: The aim of this step is to achieve adequate selectivity (peak spacing). The mobile phase and stationary phase compositions need to be taken into account. To select these, the nature of the analytes must be considered.

Once the analyte types are identified, the relevant optimization parameters may be selected. The optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization.

Step-4 System Parameter Optimization: This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factors or selectivity.

Step-5 Method Validation: Proper validation of analytical methods is important for pharmaceutical analysis when insurance of the continuing efficacy and safety of each batch manufactured relies solely on the determination of quality. The ability to control this quality is dependent upon the ability of the analytical methods, as applied under well-defined conditions and at an established level of sensitivity, to give a reliable demonstration of all deviation from target criteria. The defined validation parameters by the ICH and other regulatory bodies are:

(a) Specificity
(b) Linearity and Range
(c) Precision
(d) Accuracy
(e) Robustness
(f) Solution Stability
(g) System Suitability

1.5 Drug Profile:

1.5.1: Aspirin:

Acetyl derivative of salicylic acid.

Chemical formula: $C_9H_8O_4$

Molecular weight: 180.16

IPUAC name: 2-acetoxybenzoic acid

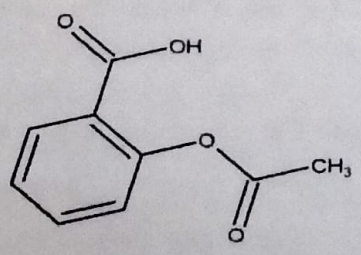

Figure 3: Aspirin

Melting point: 135.0°C

Log P: 1.19

pKa: 3.15 at 25°C

Physical state: odouless white crystals or crystalline powder with slidhtly bitter taste

Solubility: limited solubility in water , more soluble in ethanol , chloroform, ethyl ether , sodium hydroxide and sodium carbonate

Stability: Stable in dry air , hydrolysed in moist air to salicylic acid and acetic acid

Drug class: Non steroid anti inflammatory drug(NSAID)

Dosage: 0.3-1 g repeated every 4 hrs to maximum 4 g per day.

Protein binding: 80-90 %

Metabolism: In liver some amount is hydrolysed to salicylate in gut wall

Oral adsorption: Both in stomache and small intestine

Excretion: through kidney

Pharmacology: Aspirin disturbes the production of prostaglandins throughout the body by targeting cyclooxgenase 1 and cyclooxygenase 2

1.5.2: Rosuvastatin calcium:

It is a lipid-lowering drug that belongs to the statin class of medications.

Used to lower the risk of cardiovascular disease and manage abnormal lipid levels.

Chemical formula : $C_{22}H_{28}FN_3S$

Molecular weight 481.538

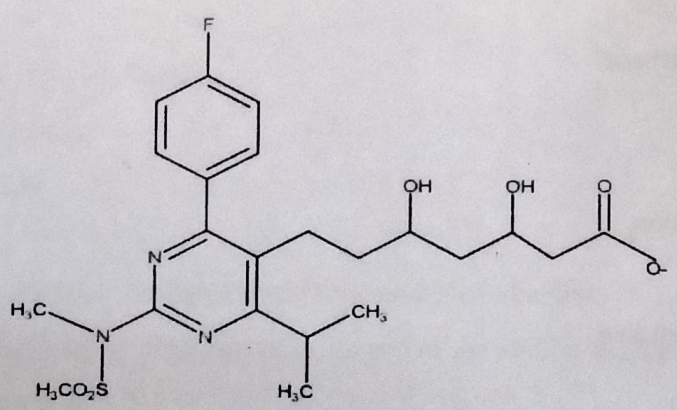

Figure 4: Rosuvastatin calcium.

IUPAC Name

(3R, 5S, 6E)-7-[4-fluorophenyl)-2-(N-methylmethanesulfanomido)-6-(propan-2-yl)pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid.

Mechanism of Action

- Rosuvastin calcium is a statin medication and a competitive inhibitor of the enzyme HMG-CoA (3-hydroxy-3-methylglutary coenzyme A) reductase, which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholestrol biosynthesis.
- Rosuvastatin calcium acts primarily in the liver, where decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increases hepatic uptake of LDL.
- Rosuvastatin calcium also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL.

Uses

- To help lower "bad" cholesterol and fats.
- Prevent strokes and heart attacks

Absorption

- Through intestine
- Bioavailability is 20%

Distribution

Rosuvastatin calcium is approximately 134 litres

Metabolism

Liver CYP2C9 (major) and CYP2

Elimination

- 28% by renal
- 72% by hepatic

Toxicity

- Myalgia
- Constipation
- Asthenia
- Abdominal pain
- Nausea

1.5.3: Clopidogrel bisulfate:

Clopidogrel bisulfate is a prodrug of a platelet inhibitor used to reduce the risk of myocardial infraction and stroke.

Chemical formula: $C_{16}H_{16}ClNO_2S$

Molecular weight : 321.822

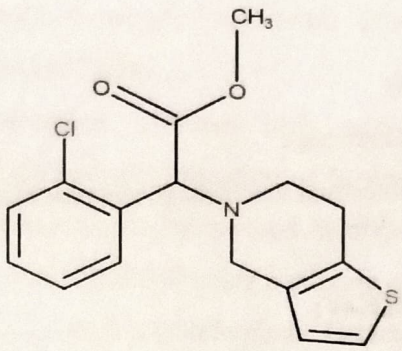

Figure 5: Clopidogrel bisulfate.

IUPAC name

methyl (2S)-2-(2-chlorophenyl)-2-{4H,5H, 6H, 7H-thieno[3, 2-c]pyridine-5-yl}acetate.

Mechanism of Action

- Clopidogrel bisulfate is metabolized to its active form by carboxylesterase-1. The active form is a platelet inhibitor that irreversibly binds to $P2Y_{12}$ ADP receptors on platelets. This binding prevents ADP binding to $P2Y_{12}$ receptor, activation of the glycoprotein GPIIb/IIIa complex, and platelet aggregation.

Uses

- Prevent heat attacks
- Unstable angina
- Antiplatelet

Absorption

- Absorbed through intestine

Volume of Distribution

- 39240±520L

Metabolism

- Prodrug absorbed through intestine and activate in the liver
- The conversion of clopidogrel to its active metabolite requires two sequential oxidative steps.

Elimination

- 50% in urine
- 40% in feces
- Remain clopidogrel irreversible bound to platelets for 11 days

Toxicity

- Vomiting
- Breathing difficulty
- Gastrointestinal hemorrhage
- Prostration gastrointestinal hemorrhage and prostration

LITERATURE REVIEW:

Telrandhe, 2013 reported the method development and validation by RP-HPLC method of the rosuvastatin calcium and Clopidogrel bisulfate. The method is a simple, accurate, specific, precise, reproducible and sensitive. The λ max of ROSU and Clop was found to be 240 nm. Coefficient correlation 0.999, Beer's Law limit 50-150 µg/ml, from the four trial of different concentration of mobile phase was selected Methanol: Water 80:20 v/v, pH 3.0 at 240 nm, flow rate 1 ml/min.

In 2013, Baliram *et al.* reported a rapid, precise, specific, accurate and simple UV spectrophotometric method was developed and validated for the estimation of Rosuvastatin calcium and clopidogrel bisulfate in combined pharmaceutical dosage forms and laboratory prepared mixtures. The method was validated in terms of linearity, accuracy, prevision, and specificity, limit of detection (LOD) and limit of Quantitation (LOQ) as per ICH guidelines. To overcome the problem of spectral interference first order derivative spectroscopic method were adopted. The method were adopted. The method obeys Beer's Law in concentration ranges of 2-10 µg/ml for both drug. The λmax of Rosuvastatin calcium and Clopidogrel bisulfate were found to be at 244 nm and 219 nm respectively.

In 2013, Sultana *et al.* described two methods for the simultaneous determination of clopidogrel bisulfate with HMG Co-A reductase inhibitors/statins. The first method comprised of quantitative determinations of clopidogrel bisulfate in presence of HMG Co-A reductase inhibitors (atorvastatin, pravastatin simvastatin and rosuvastatin) in buffers of pH 1, 4 (simulating gastric environments), pH 7.4 (simulating blood pH) and pH 9 simulating intestinal pH) at body temperature (37 °C) using UV/VIS spectrophotometer).

In 2010, Chaudhary *et al.* describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of losartan potassium and Perindopril Erbumine in tablet formulation. Chromatography was performed on a ODS Hypersil C18 (250mm×4.6mm i.d., 5 □m particle size) column with mobile phase containing Acetonitrile: Acidic Water pH 3.4 (50+50). The flow rate was 1.5 mL/min and the eluent was monitored at 218nm.

In 2012, Tank *et al.* described validated high performance thin layer liquid chromatographic (HPTLC) method for estimation of Rosuvastatin calcium (Rosu) and Aspirin (ASP) in capsule dosage form. The method involved separation of components by TLC on a precoated silica gel 60 dF254 using a mixture of n-Hexane: Acetone: Ethyl acetate: Formic acid (6:3:1:0.2 v/v) as a mobile phase. Detection of spots was carried out at 240nm for Rosuvastatin calcium and Aspirin both.

In 2018, Pisal *et al.* develop RP-4PLL method for the simultaneous estimation of aspirin, for Rosuvastatin and clopidogrel in bulk and pharmaceutical dosage form. The specification of chromatographic system, HPLC (18 column 4.6×250mm. 5µm with mobile phase containing water at pH 2.51 with 0.1% v/v i.d. orthophosphoric acid, acetonitrile in the ration 5:50 Rosuvastatin 1ml/min. The retention time of aspirin rosuvastatin and clopidogrel was 4.3 mm, 7.6 min and 16.6min respectively.

In 2018, Saeed *et al.* develop an easy and precise metod for the estimation of Aspirin impurities from salicylic acid and heavy metals in the Apsirin tables available in market using HPLC, UV-VIs spectrophotometry and atomic elongation spectrophotometic methods. HPLC was carried out by using (18 column as stationary phase and acetonitrile : water in ration (10:90 v/v) as mobile phase for HPLC method and as a solvent for U.V. VIS spectrophotometery. Aspirin and Salicylic acid gave assurance maximum at 226 and 296nm in CAN: H_2O solvent. The Beer's Law was obeyed in the range 0.05 -20 for ASP and 0.02-8 µg/ml for SALK. For HPLC LOD value was 0.006 for ASP and 0.004 µg/ml for SAS. The % recovery for the developed method was found 98.80 to 101.26% for ASP and 98.67 to 103.33% for SAL which are in the acceptable range.

In 2018, Pajak *et al.* develop a new simple and cost effective. TLC densitometric method for the simultaneous quantitative determining of acetylsalicylic acid and ascorbic acid in combined tablets form. Separation was performed on a aluminium silica gel $60F_{254}$ plates using chloroform ethanol: glacid acid at a volume ratio of 5:4: 0.03 as mobile phase. Developed method has good sensitivity with low value of LoD and LOQ of both examined substances. The linearity range was found to be 1.50-9.00 µg/spot and 1.50-

13.50 µg/spot for acetlysalicylic acid and ascorbic acid respectively. Coefficient of variation was less than 3% confirms the accuracy and precision of the proposed method.

In 2016, Jadhav *et al.* develop an easy simple specific, speedy precise and accurate UV spectrophotometric method for the content determination of Telmisartan. The developed method was linear for a range of 5 µg/ml-25 µg/ml with correlation coefficient of 0.9994. The LOD and LOQ of telmisartan was found to be 0.3 µg/ml and 4.5 µg/ml respectively.

In 2015, Madhukar *et al.* developed a rapid, simple and precise RP-HPLC method for the simultaneous analysis of hydrochlorothiazide (HTZ), Amlopidine besylate (AML) and Telmisartan hydrochloride (TLM) in a tablet dosage form. This method was performed with a thermosil C_{18} (4.6 -100, 3.7) column with 40:60 (v/v) 20mm potassium dihydrogen orthophosphate buffer: methanol as mobile phase at a flow rate of 1.0ml/min UV detection at 248nm. The retention times of HTZ, AML, TLM were 1.823, 2.638 and 4.198 min respectively. Calibration curve plots were linear over the con. Ranges 6.25-100mg/ml for HTZ, 25-40 µg/ml for AML an 20.320 µg/ml for TLM. Limit of detection were 0.004, 0.0016 and 0.0128 µg/ml and limit of quantification were 0.013, 00052 and 0.0416 µg/ml for HTM, AML and TLM respectively.

In 2019, Tiwari *et al.* developed a new simple, precise, rapid and linear RP-HPLC method for simultaneous qualitative and quantitative estimation of Rosuvastatin (Ros), Clopidogrel (Clop) and Aspirin in dosage form. A good chromatography separation was achieved by isocratic method using a hypersil BDS C_{18} column (250mm×4.6,5□m) and a mobile phase containing of KH_2PO_4 buffer pH-6.0: acetonitrile in the ratio 60:40 at a flow rate of 1ml/min. The effluents were monitored at 242 nm with UV-visible detector. The calibration curves obtained were linear over the concentration range of 7.5-22.5 µg/ml and 1-3µg/ml for Clop, ASP and ROS respectively. The rune time is 7.0 minute for each sample. The retention time of ASP, CLOP, ROS was 3.103 min, 4.277 min and 5.707 min respectively. The high recovery value (99% -101%) indicate the accuracy and low% RSD made study precise. So this developed method can be used for routine monitoring of CLOP, ASP & ROS.

Croitoru, 2015 reperted a simple sensitive and specific reverse phase liquid chromatographic method was developed and validated for simultaneous quantification of Clopidogrel it carboxylic acid metabolite and atorvastatin in human serum. Plasma samples were deproteinized with acetonitrile and Ibuprofen was chosen as internal standard. Chromatographic separation was performed o an C_{18} column (250×4.5nm,

5μm) via gradient elution with mobile phase consisting of 10mm phosphoric acid (sodium) buffer solution at pH 206 adjusted with 85% orthophosphoric acid); acetonitrile methanol with flow rate of ml/mm. Detection was at 220nm with PDA detector. The developed method was validated in terms of linearity sensitivity, precision, accuracy, limit of quantification and stability test. Calibration curves of the analytes were found to be linear in the range of 0.008-2μg/ml for Clopidogrel, 0.01-4.0 $\mu g/ml^{-1}$ for its carboxylic acid metabolite and 0.005-2.5$\mu g/ml^{-1}$ for atorvastatin.

In 2018, Sivagamic *et al.* developed and validated a simple, accurate, precise and economical method for the simultaneous estimation of the clopidogrel and Atorvastatin in table dosage form law RP-HPLC method. The chromatogram was run through standard C_8 (250×4.6, 5μm) column. The mobile phase containing buffer 0.1% potassium Dihydrogen phosphate: Acetonitrile taken in the ratio 45:55 was pumped through column at a flow rate of 1ml/min pH was adjusted to 3.8 with dil. Orthophosphoric acid solution. Temperture was maintained at 30 °C optimized wavelength selected for Clopidogrel and Atorvastatin was 247nm. Retention time of Clopidogrel and Atorvastatin were 2.553 min and 3.224 min respectively. The calibration curves were linear in the concentration rate 0.150μg/,l for the drug. The correlation coefficient was 0.999. The percentage array in formulation was found to be 99.60% and 99.14% for Clopidogrel and Atorvastatin respectively. Percentage RSD of the Clopidogrel and Atorvastatin were 0.4 and 0.3 respectively. The developed and validated method can be used for the routine analysis of Clopidogrel and Atorvastatin in API and pharmaceutical dosage form.

In 2015, Azizramdan *et al.* developed a simple, direct and accurate spectrophotometric method has been developed for the determination of rosuvastatin in pure and pharmaceutical formulation by complex formation with bromocresol green. The method involves the formation of a yellow ion pair complex between rosuvastatin and bromocresol green (BCG) reagent in chloroform. The formed complex was measured at □max 416nm against the reagent blank prepared in the same manner.

In 2020, Nallavelli *et al.* intended to improve the array strategy for simultaneous estimation of telmisartan and atorvastatin by using HPLC, mobile phase of methanol water and 250nm detection wavelength. The retention time were 2.4 minutes for telmisartin and 3.0 minutes for atorvastatin. The analytical technique showed linearity over the range 20 to 80 ppm of the target concentration to both the drugs. The recuperation was 99.9% for both the drugs. The planned method was found to be simple,

explicit, linear, specific and rugged. Hence, it can be used for routine analysis of telmisartan and atorvastatin in bulk and pharmaceutical formulations.

RESEARCH GAP IDENTIFICATION: The literature review reveals that several works has been carried out on the quantification and analysis of antihypertensive drugs including HPLC, dissolution tests, spectorphotometric methods. The present study will describe the development of a new, rapid, cost-effective, sensitive and reproducible HPLC method for the multi-component analysis of some important categories of antihypertensive agents. That offers certain advantages in its simplicity and sensitivity and applicable in routine analysis.

OBJECTIVES: Main objectives of the proposed study are as:

- Analytical method development and validation for the multi-component analysis of some important categories of anti-hypertensive agents.
- To develop and validate analytical method for simultaneous estimation of anti-hypertensive drugs in pharmaceutical dosage form.
- Development and validation of methods as per ICH Q2 (R1) for newer combinations help to detect drug substances present in diluent and recipients.

PROPOSED METHODOLOGY:

Mentioned API Active Pharmaceutical ingredients to be used in Research Work:

Drugs
Aspirin
Clopidogrel Bisulphate
Rosuvastatin Calcium

Reagents to be used for method development and validation:

Orthophosphoric acid
Acetonitrile , HPLC Grade
HPLC Water
Mobile Phase
Standard- Aspirin,Rosuvastatin,telmisaartan and clopidogrel NIST Traceable
Test sample: Dosage forms with API Aspirin- 75/150 mg , Rosuvastatin- 10/20mg,

Clopidogreol- 25/150 mg , Telmisartan- 40/80mg per dosage form.

Equipments:

Liquid chromatograph equipped with autosampler and UV Detector at 237 nm
Column: C18 5µ , 250×4.6 mm or equivalent
Volumeteric flask
Volumetric pipette
Analytical Balance
Sonicator

General Method:

- The various components will be separated from each other as well as well as from other components in the sample by HPLC using a reverse phase column of suitable size. The components will be detected using an UV detector and quantitated by a method of external standardization.
- Developed method will be validated for its intended use to determine the accuracy and repeatability of method as per the ICH guidelines. Various validation parameter are: accuracy, precision, linearity and range, limit of detection, robustness and specificity.

THESIS OUTLINE: Thesis will comprise five chapters as:

Chapter 1: Introduction - includes brief about causes of hypertension , antihypertensive drugs , significance of analytical methods , high performance liquid chromatography and various steps involved in method development of HPLC and validation. It also includes drud profile of API's used in study,

Chapter 2: Literature Review - The technique of chromatography was originally developed by Russian botanist M.S.D Tswett in 1903 but after that plenty of revolutions and amendments were done and it is still going on. HPLC considering an advanced form of liquid chromatography used in seperating the complex misture of molecules encountered in chemical and biological system. More than 85 percent of general pharmaceuticals are analysed by HPLC.

Chapter 3: Materials and Methods - The various components /API's will be separated from each other as well as from other components in the sample by HPLC using reverse

phase column . The components will be detected using UV detector and then quantified by a method of external standardization. Developed method will be validated as per ICH guidelines.

Chapter 4: Results and Discussion - This chapter deals with the results/outcomes of the method development and validation parameters.

Chapter 5: Summary and Conclusions – This chapter will comprise summary of entire study according to objectives along with conclusions. This chapter also include the future scope of the research.

Chapter 6: References – All the references will be written as per Harvard Guidelines.

References:

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This is to certify that the Synopsis for /Ph.D. Thesis titled, **"Analytical Method Development and Validation for the Multi-component Analysis of Some Important Categories of Anti-hypertensive Agents",** submitted by **Ms. Monika Puri** to the Career Point University, Hamirpur (H.P.), India in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the Department of Chemistry, School of Basic and Applied Sciences has been approved by the Research Advisory Committee (RAC) after an Oral Examination of the student on dated 22/07/2021.

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